

# Neutrophil Function after Exposure to Polychlorinated Biphenyls *in vitro*

Patricia E. Ganey,<sup>1,2,3</sup> Jay E. Sirois,<sup>1</sup> Michael Denison,<sup>3,4</sup> J. Paul Robinson,<sup>5</sup> and Robert A. Roth<sup>1,3</sup>

<sup>1</sup>Department of Pharmacology and Toxicology, <sup>2</sup>Department of Medicine, <sup>3</sup>Institute for Environmental Toxicology, and <sup>4</sup>Department of Biochemistry, Michigan State University, East Lansing, MI 48824 USA; <sup>5</sup>Purdue University Cytometry Laboratories and Department of Veterinary Physiology and Pharmacology, Purdue University, West Lafayette, IN 47906 USA

Polychlorinated biphenyls (PCBs) are known to be immunotoxic, yet the effects on neutrophil (PMN) function are not well characterized. We incubated PMNs isolated from rat peritoneum with a mixture of PCB congeners, Aroclor 1242, in the absence or presence of either phorbol myristate acetate (PMA) to stimulate generation of superoxide anion ( $O_2^-$ ) or N-formyl-methionyl-leucyl-phenylalanine (fMLP) to induce degranulation (measured as release of  $\beta$ -glucuronidase). Aroclor 1242 alone stimulated  $O_2^-$  production at a concentration of 10  $\mu$ g/ml. Significant cytotoxicity was not observed under these conditions. This concentration of Aroclor 1242 also increased  $O_2^-$  generation in PMNs activated with 20 ng PMA/ml. In the presence of a concentration of PMA (2 ng/ml) that by itself did not stimulate production of  $O_2^-$ , 1  $\mu$ g Aroclor 1242/ml caused significant generation of  $O_2^-$ , indicating synergy between Aroclor 1242 and PMA. Aroclor 1242 caused release of  $\beta$ -glucuronidase from quiescent PMNs; however, in PMNs stimulated with fMLP to undergo degranulation, Aroclor 1242 inhibited release of  $\beta$ -glucuronidase. The effects of two PCB congeners, one that binds to the Ah receptor (3,3',4,4'-tetrachlorobiphenyl) and one that has little affinity for this receptor (2,2',4,4'-tetrachlorobiphenyl) were examined. 3,3',4,4'-Tetrachlorobiphenyl had no effect on PMN function *in vitro*, whereas 2,2',4,4'-tetrachlorobiphenyl had effects similar to those observed with Aroclor 1242. These results indicate that PCBs affect PMN function *in vitro* in a complex manner, stimulating or inhibiting function under different conditions. These effects are apparently not mediated through the Ah receptor. **Key words:** Ah receptor, degranulation, neutrophils, polychlorinated biphenyls, superoxide anion. *Environ Health Perspect* 101:430–434 (1993)

Polychlorinated biphenyls (PCBs) are persistent environmental contaminants. Exposure to PCBs is associated with a variety of biological effects including induction of enzymes involved in xenobiotic metabolism, alterations in reproductive function, hepatotoxicity, carcinogenicity, and dermal lesions. Thymic atrophy is a consistent finding in PCB-treated animals, and alterations in immunity after exposure to PCBs have been reported (1). For example, monkeys fed Aroclor mixtures of PCBs for 11 months had lower anti-sheep red blood cell

titers and decreased concentrations of  $\gamma$ -globulin compared to controls (2). The splenic plaque-forming cell response to sheep red blood cells was suppressed in mice exposed acutely to Aroclor mixtures of PCBs (3) or to the coplanar PCB congener 3,3',4,4'-tetrachlorobiphenyl (1). In humans accidentally exposed to PCBs, decreases in the total number of T-lymphocytes (4) and in the percentage of peripheral T-lymphocytes (5), as well in the concentrations of IgM and IgA in serum (5), were reported.

Although effects of PCBs on cell-mediated and humoral immunity have been investigated, little is known about the effects of PCBs on polymorphonuclear leukocytes (PMNs), which contribute to nonspecific immunity. One study reported that 3,3',4,4'-tetrachlorobiphenyl decreased generation of leukotriene  $B_4$  from human PMNs stimulated with sodium fluoride (6). Because sodium fluoride activates G-proteins, which results in metabolism of arachidonic acid and subsequent formation of leukotrienes, the authors speculated that the interaction of sodium fluoride and PCBs with PMNs induced synthesis of a set of inhibitory G-proteins that reduced leukotriene formation. Interestingly, in the same study, exposure to 3,3',4,4'-tetrachlorobiphenyl increased leukotriene  $B_4$  release from opsonized zymosan-stimulated PMNs and from PMNs pretreated with sodium fluoride, suggesting that PCBs enhance the generation of leukotrienes once production has been initiated. Thus, one function of PMNs is either inhibited or augmented, depending on the experimental conditions. To extrapolate this observation to possible consequences if this happened *in vivo*, suppression of PMN function by PCBs could lead to increased susceptibility to infection. Alternatively, activation of PMNs has been implicated in tissue injury in a variety of disease models.

To begin to explore possible alterations in PMN function caused by PCBs, the effect of *in vitro* exposure to Aroclor 1242, a complex mixture of PCB congeners, on PMN function was evaluated. Some biological effects of PCBs, for example, induction of hepatic aryl hydrocarbon hydroxylase activity (7), are mediated through

binding to the Ah receptor, a cytosolic receptor that binds to PCBs. The resultant receptor–ligand complex translocates to the nucleus and alters gene expression to initiate toxic and other biologic effects. Binding to the Ah receptor and biological activity depend on the structure of individual congeners, such that coplanar PCB congeners (e.g., 3,3',4,4'-tetrachlorobiphenyl) have the greatest affinity for the receptor (7). To test the potential role of binding to the Ah receptor in initiating alterations in PMN function produced by Aroclor 1242, we examined the effects of two PCB congeners, one which binds with high affinity to the Ah receptor and one that has little affinity for the receptor (8).

## Materials and Methods

Aroclor 1242, 2,2',4,4'-tetrachlorobiphenyl (>99% pure), and 3,3',4,4'-tetrachlorobiphenyl (>99% pure) were purchased from ChemService (West Chester, Pennsylvania). Phorbol myristate acetate (PMA) was purchased from LC Services (Woburn, Massachusetts), and superoxide dismutase (SOD) was obtained from Diagnostic Data, Inc. (Mountainview, California). We obtained [ $^3H$ ]-2,3,7,8-tetrachlorodibenzo-*p*-dioxin ([ $^3H$ ]TCDD) and 2,3,7,8-tetrachlorodibenzofuran, used in determining the presence of Ah receptors in rat PMNs, from Stephen Safe (Texas A & M University). All other chemicals were of the highest grade commercially available.

We isolated glycogen-elicited PMNs from the peritoneum of male Sprague-Dawley, retired breeder rats [CD-Crl:CD (SD)BR VAF/Plus; Charles River Laboratories, Portage, Michigan], as described previously (9). Briefly, 30–40 ml of 1% glycogen in sterile saline was injected into the peritoneum of rats anesthetized with diethylether. Four hours later the rats were anesthetized again with diethylether and were killed by decapitation. We rinsed the peritoneum with 30 ml of 0.1 M phosphate-buffered saline (PBS) containing 1 U/ml heparin. The rinse solution was filtered through gauze and centrifuged for 7 min at 500g. We lysed contaminating red blood cells in 15 ml of 0.15 M  $NH_4Cl$ , and then centrifuged the PMNs for 7 min at 300g. Cells were washed once with PBS,

Address correspondence to P.E. Ganey, Department of Pharmacology and Toxicology, Life Sciences Building, Michigan State University, East Lansing, MI 48824 USA. This work was supported by grant ES04911 from NIEHS. R.A.R. was supported in part by a Burroughs Wellcome Toxicology Scholar Award. We thank Maria Colligan, Dianne Schwartz, and Eric Shobe for excellent technical assistance.

Received 2 February 1993; accepted 21 June 1993.

then resuspended in Hanks' balanced salt solution (HBSS). The final concentration of PMNs in the assays was  $2 \times 10^6$  cells/ml. The percentage of PMNs in the cell preparation was routinely >95%, and viability (i.e., ability to exclude trypan blue dye) was  $\geq 95\%$ .

PMNs were stimulated with either N-formyl-methionyl-leucyl-phenylalanine (fMLP) in the presence of cytochalasin B (5  $\mu\text{g}/\text{ml}$ ) or with PMA. Concentrations of fMLP and PMA were chosen based on previous studies in which these concentrations were shown to stimulate degranulation (9) or superoxide anion production (10), respectively.

For measurement of superoxide anion ( $\text{O}_2^-$ ) generation, Aroclor 1242 was dissolved in methanol, 3,3',4,4'-tetrachlorobiphenyl was dissolved in dimethylformamide, and 2,2',4,4'-tetrachlorobiphenyl was dissolved in 50% acetone/50% dimethylsulfoxide. We added 1  $\mu\text{l}$  of the stock solutions to 1 ml of HBSS containing the suspended PMNs to achieve the desired final concentrations. Control PMNs received an equivalent volume of the appropriate vehicle. Preliminary studies indicated that none of the vehicles affected  $\text{O}_2^-$  production at the concentrations used.

We incubated PMNs at 37°C with PCBs for 30 min. PMA was then added, and samples were incubated for an additional 10 min. We measured  $\text{O}_2^-$  generated during this 40-min period spectrophotometrically as the SOD-sensitive reduction of ferricytochrome C (11). For every sample, two tubes were incubated: one to which SOD (85 U/ml) was added before incubation and one to which SOD was added after incubation. We used the difference in absorbance (550 nm) of the cell-free supernatant fluids from these two tubes to estimate the amount of cytochrome C reduced, using an extinction coefficient of  $18.5 \text{ cm}^{-1} \text{ mM}^{-1}$ .

To study the degranulation response, PMNs were incubated with PCBs at 37°C in the presence of cytochalasin B for 10 min. We then added fMLP and incubated the cells for another 10 min. The activity of  $\beta$ -glucuronidase in the cell-free supernatant fluids was measured as the release of phenolphthalein from its glucuronide at pH 4.5 during incubation at 37°C (12). We determined total  $\beta$ -glucuronidase activity in PMNs lysed with 1% Triton X-100 and sonication, and values are presented as the percentage of total  $\beta$ -glucuronidase released into the buffer. The presence of PCBs did not interfere with determination of the activity of  $\beta$ -glucuronidase.

We estimated cytotoxicity from release of the cytosolic enzyme lactate dehydrogenase (LDH). PMNs were incubated with PCBs in the absence or presence of PMA

as described above for measurement of generation of  $\text{O}_2^-$ . We determined the activity of LDH in the cell-free supernatant fluids according to the method of Bergmeyer and Bernt (13). A separate aliquot of PMNs was lysed with Triton X-100 and sonication, and total LDH activity was determined in the cell-free supernatant fluids from these lysates. Cytotoxicity is expressed as the percentage of total LDH activity released into the buffer. The presence of PCBs did not interfere with determination of the activity of LDH.

Specific binding of [ $^3\text{H}$ ]TCDD to cytosol from rat peritoneal PMNs was determined using the hydroxylapatite binding assay (14). Receptor concentrations are presented as femtomoles of [ $^3\text{H}$ ]TCDD specifically bound per milligram of protein.

Results are presented as means  $\pm$  SEM. For all results presented, *n* represents the number of repetitions of an experiment, and each experiment used cells from different rats. Data were analyzed by analysis of variance. We transformed data that did not satisfy the criterion of homogeneity before further statistical analysis. Individual means were compared using the Student-Newman-Keuls' test. For all studies, the criterion for significance was  $p \leq 0.05$ .

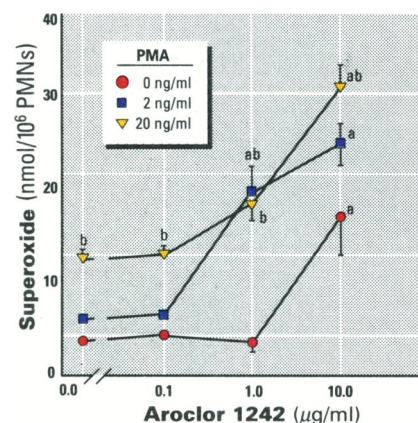
## Results

Quiescent PMNs produced no  $\text{O}_2^-$ , and incubation with Aroclor 1242 up to 1  $\mu\text{g}/\text{ml}$  did not stimulate generation of  $\text{O}_2^-$  (Fig. 1). When PMNs were exposed to Aroclor 1242 at a concentration of 10  $\mu\text{g}/\text{ml}$  for 30 min, a significant amount of  $\text{O}_2^-$  was produced.

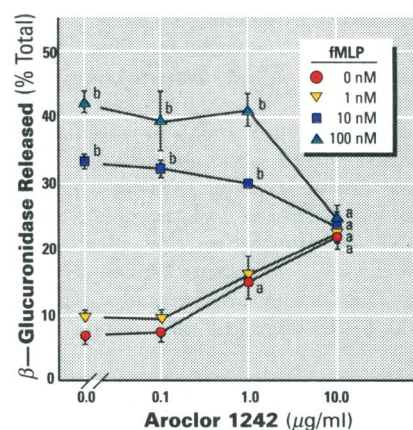
Incubation of PMNs with 20 ng PMA/ml, but not 2 ng PMA/ml, caused significant generation of  $\text{O}_2^-$  compared to quiescent PMNs. A significant increase in  $\text{O}_2^-$  generation was observed at 1 and 10  $\mu\text{g}/\text{ml}$  Aroclor 1242 when PMNs were treated with 2 ng PMA/ml. In PMNs activated with 20 ng PMA/ml, effects of Aroclor 1242 were similar to those seen in the absence of PMA: increased production of  $\text{O}_2^-$  was observed only at 10  $\mu\text{g}$  Aroclor 1242/ml.

About 7% of the total  $\beta$ -glucuronidase activity appeared in the medium above quiescent PMNs (Fig. 2). Incubation with Aroclor 1242 at concentrations of 1  $\mu\text{g}/\text{ml}$  and higher increased release of  $\beta$ -glucuronidase from PMNs.

In the absence of Aroclor 1242, a concentration-related increase in the percentage of  $\beta$ -glucuronidase released by control PMNs was observed with fMLP stimulation (Fig. 2). Exposure to Aroclor 1242 did not affect release of  $\beta$ -glucuronidase from PMNs stimulated with 1 nM fMLP. In PMNs stimulated with higher concentrations of fMLP, Aroclor 1242 (10  $\mu\text{g}/\text{ml}$ )



**Figure 1.** Superoxide anion generation by PMNs exposed to Aroclor 1242. Glycogen-elicited peritoneal PMNs ( $2 \times 10^6/\text{ml}$ ) were incubated for 30 min in the presence of Aroclor 1242 (at the concentrations indicated) or an equivalent volume of vehicle (methanol), followed by an additional 10-min incubation with PMA.  $\text{O}_2^-$  produced over this 40-min period was determined as described in Materials and Methods ( $n = 4-6$ ). <sup>a</sup>Significantly different from respective value with 0  $\mu\text{g}$  Aroclor 1242/ml; <sup>b</sup>significantly different from respective value with 0 ng PMA/ml.

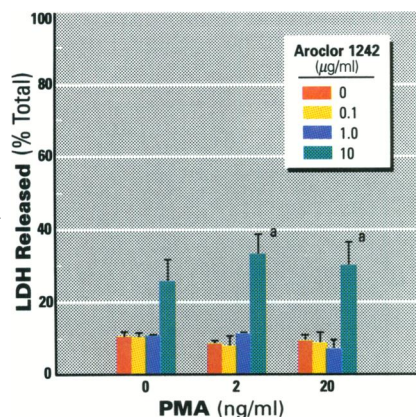


**Figure 2.** Release of  $\beta$ -glucuronidase from PMNs exposed to Aroclor 1242. Glycogen-elicited peritoneal PMNs ( $2 \times 10^6/\text{ml}$ ) were incubated for 10 min in the presence of Aroclor 1242 (at the concentrations indicated) or an equivalent volume of vehicle, followed by an additional 10-min incubation with fMLP. Cytochalasin B (5  $\mu\text{g}/\text{ml}$ ) was present in all samples. Activity of  $\beta$ -glucuronidase released into the medium was determined as described in Materials and Methods. Total  $\beta$ -glucuronidase activity was  $1.36 \pm 0.11 \text{ U}/10^6 \text{ PMNs}$  ( $n = 3-5$ ). <sup>a</sup>Significantly different from respective value with 0  $\mu\text{g}$  Aroclor 1242/ml; <sup>b</sup>significantly different from respective value with 0 nM fMLP.

inhibited degranulation in response to fMLP.

LDH release was increased when PMNs were incubated with 10  $\mu\text{g}$  Aroclor 1242/ml (Fig. 3). These values reached statistical significance only when PMNs were co-incubated with PMA.

Because some effects of PCBs are mediated through the Ah receptor, it was of



**Figure 3.** Release of lactate dehydrogenase (LDH) from PMNs exposed to Aroclor 1242. PMNs were incubated as described in the legend to Figure 1, and LDH activity released by the PMNs was determined as described in Materials and Methods. Total LDH activity was  $85 \pm 34$  U/ $10^6$  PMNs ( $n = 4-6$ ). <sup>a</sup>Significantly different from respective value with 0 µg Aroclor 1242/ml.

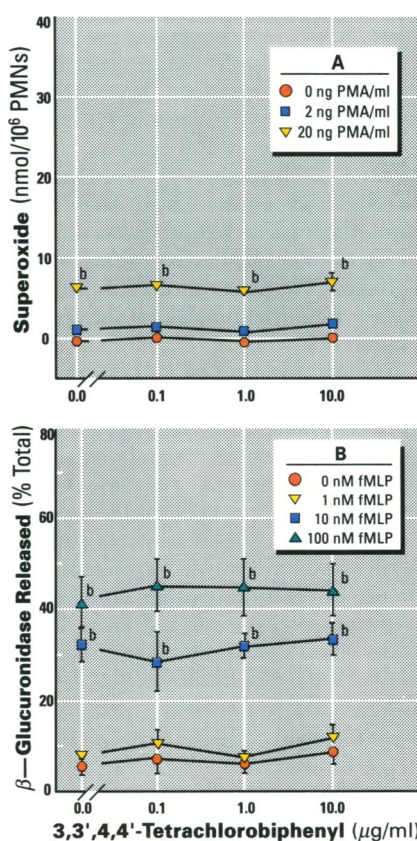
interest to determine the presence and concentration of the Ah receptor in rat PMNs. In cytosol from glycogen-elicited PMNs,  $14.2 \pm 2.5$  fmol [ $^3$ H]TCDD/mg protein were specifically bound. This value is consistent with that reported for mouse peritoneal PMNs (18–25 fmol/mg protein) (15).

Exposure to 3,3',4,4'-tetrachlorobiphenyl did not affect generation of  $O_2^-$  in the absence or in the presence of PMA (Fig. 4A). Release of  $\beta$ -glucuronidase from PMNs was also unaffected by incubation with 3,3',4,4'-tetrachlorobiphenyl (Fig. 4B). This lack of effect was observed in both the absence and the presence of fMLP. LDH release by PMNs was unaffected by exposure to 3,3',4,4'-tetrachlorobiphenyl (Fig. 5).

In the absence of PMA, 2,2',4,4'-tetrachlorobiphenyl did not stimulate production of  $O_2^-$  (Fig. 6A). In the presence of PMA, concentrations of 2,2',4,4'-tetrachlorobiphenyl of 1 and 10 µg/ml increased generation of  $O_2^-$ .

In the absence of fMLP, 2,2',4,4'-tetrachlorobiphenyl did not significantly affect release of  $\beta$ -glucuronidase from PMNs (Fig. 6B). Beta-glucuronidase release from PMNs exposed to 2,2',4,4'-tetrachlorobiphenyl was not affected by addition of fMLP at a concentration of 1 nM. When PMNs were activated with higher concentrations of fMLP, exposure to 2,2',4,4'-tetrachlorobiphenyl (1 or 10 µg/ml) inhibited the fMLP-stimulated release of  $\beta$ -glucuronidase.

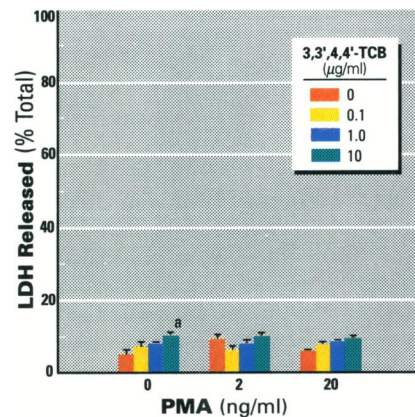
Exposure to 10 µg 2,2',4,4'-tetrachlorobiphenyl/ml increased release of LDH, and this was only statistically significant when PMNs were also exposed to PMA (Fig. 7).



**Figure 4.** Superoxide anion generation (A) and release of  $\beta$ -glucuronidase (B) by PMNs exposed to 3,3',4,4'-tetrachlorobiphenyl. Experiments were performed as described in the legends to Figure 1 and 2. The vehicle used for controls was dimethylformamide. Total  $\beta$ -glucuronidase activity was  $1.10 \pm 0.07$  U/ $10^6$  PMNs ( $n = 5$ ). <sup>b</sup>Significantly different from respective value without PMN stimulus.

## Discussion

In the present study, the function of rat PMNs was affected by exposure to PCBs *in vitro*. Aroclor 1242 stimulated  $O_2^-$  generation in a dose-related manner (Fig. 1). Production of  $O_2^-$  by PMA-activated PMNs was also altered. Two concentrations of PMA were used in these studies: one that does not cause significant production of  $O_2^-$  (2 ng/ml), and one that does (20 ng/ml). The purpose of using the smaller concentration of PMA was to allow detection of synergy between PCBs and a known activator of PMNs. Such a synergistic effect was observed with Aroclor 1242: the concentration-response curve to Aroclor 1242 shifted to the left in the presence of 2 ng PMA/ml. This effect on  $O_2^-$  production occurred in the absence of significant cytotoxicity to the PMNs, decreasing the likelihood that the increased generation of oxygen free-radicals occurred in response to cell injury. The mechanism by which PCBs enhance  $O_2^-$  generation by PMNs is unknown. PCBs might increase  $O_2^-$  generation through effects on protein

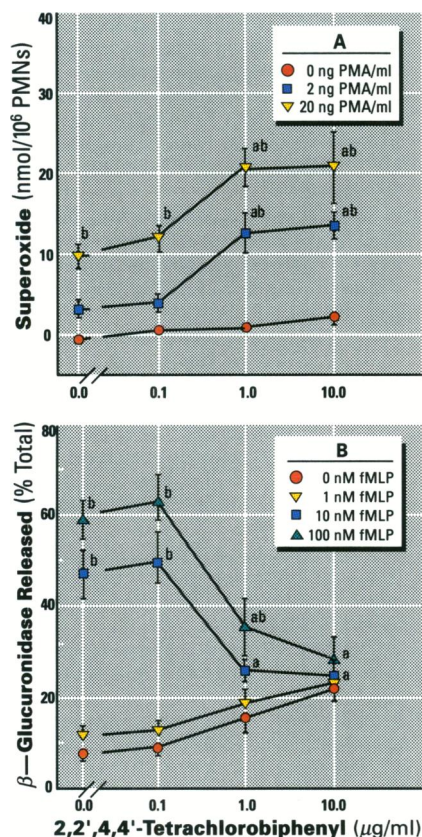


**Figure 5.** Release of lactate dehydrogenase (LDH) from PMNs exposed to 3,3',4,4'-tetrachlorobiphenyl. PMNs were incubated as described in the legend to Figure 1, and LDH activity released by the PMNs was determined as described in Materials and Methods. Total LDH activity was  $54 \pm 4$  U/ $10^3$  PMNs ( $n = 5$ ). <sup>a</sup>Significantly different from respective value with 0 µg 3,3',4,4'-tetrachlorobiphenyl/ml.

kinase C (PKC). Activation of PKC is associated with generation of  $O_2^-$  in PMNs stimulated with PMA (16), and PCBs have been reported to activate PKC directly (17–19). Alternatively, PCBs may increase the rate of generation of  $O_2^-$ .

Several reports suggest that PCBs have effects on immunity. For example, exposure to PCBs *in vivo* caused suppression of humoral immunity in mice and monkeys (1–3,20). In mice this effect was related to the chlorine content of PCB mixtures: those mixtures with a higher percentage of chlorine were more potent immunosuppressants (3). Many of the biological effects of PCBs and other halogenated aromatic hydrocarbons correlate with binding affinity for the Ah receptor (8), with TCDD having the highest affinity for the receptor. Accordingly, several of the immunotoxic effects of PCBs appear to be mediated through the Ah receptor. Treatment of mice with a coplanar congener (3,3',4,4'-tetrachlorobiphenyl) that binds to the Ah receptor with high affinity resulted in inhibition of the primary direct splenic antibody response, whereas treatment with a congener with lower affinity for the Ah receptor (2,2',5,5'-tetrachlorobiphenyl) did not alter this response (1). In addition, 3,3',4,4'-tetrachlorobiphenyl was immunotoxic in C57BL/6J mice, which are sensitive to TCDD and have high affinity Ah receptors, but not in DBA/2J mice, which have defective Ah receptors. These results suggest that the Ah receptor may play a role in producing immunotoxic effects.

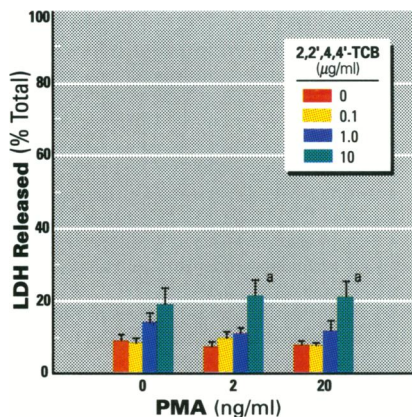
This hypothesis is supported by the observation that TCDD itself is immunotoxic. Cell-mediated immunity was sup-



**Figure 6.** Superoxide anion generation (A) and release of  $\beta$ -glucuronidase (B) by PMNs exposed to 2,2',4,4'-tetrachlorobiphenyl. Experiments were performed as described in the legends to Figures 1 and 2. The vehicle used for controls was 50% acetone/50% dimethylsulfoxide. Total  $\beta$ -glucuronidase activity was  $1.35 \pm 0.16$  U/ $10^6$  PMNs. (A)  $n = 5$ ; (B)  $n = 4$ . <sup>a</sup>Significantly different from respective value with 0  $\mu$ g 2,2',4,4'-tetrachlorobiphenyl/ml; <sup>b</sup>significantly different from respective value without PMN stimulus.

pressed by TCDD in a dose-related manner (21). Treatment of mice with TCDD resulted in decreased generation of cytotoxic T-lymphocytes (22) and suppression of differentiation of B-cells (23). In addition, decreased resistance to *Salmonella* infections has been reported (24). Alterations in PMN function have also been reported after exposure of mice to TCDD (15). B6C3F<sub>1</sub> mice are TCDD sensitive and have high-affinity Ah receptors. Acute exposure of B6C3F<sub>1</sub> mice to TCDD resulted in inhibition of PMA-stimulated, PMN-mediated cytolysis of tumor cells (15). In contrast, function was not altered in PMNs from TCDD-treated DBA/2N mice.

Unlike effects on PMNs observed after TCDD exposure of mice *in vivo* (15), our results suggest that effects observed after exposure of rat PMNs to PCBs *in vitro* are not Ah receptor-mediated. 2,2',4,4'-Tetrachlorobiphenyl has little affinity for the Ah receptor and produces a phenobarbital-like induction of drug-metabolizing



**Figure 7.** Release of lactate dehydrogenase (LDH) from PMNs exposed to 2,2',4,4'-tetrachlorobiphenyl. PMNs were incubated as described in the legend to Figure 1, and LDH activity released by the PMNs was determined as described in Materials and Methods. Total LDH activity was  $58 \pm 7$  U/ $10^3$  PMNs ( $n = 5$ ). <sup>a</sup>Significantly different from respective value with 0  $\mu$ g 2,2',4,4'-tetrachlorobiphenyl/ml.

enzymes, whereas 3,3',4,4'-tetrachlorobiphenyl binds to the Ah receptor with high affinity and causes induction of drug-metabolizing enzymes similar to that seen with TCDD (8). Effects on PMN function similar to those seen with Aroclor 1242 were observed when PMNs were exposed to 2,2',4,4'-tetrachlorobiphenyl (Fig. 6A), but 3,3',4,4'-tetrachlorobiphenyl did not alter O<sub>2</sub><sup>-</sup> production by PMNs (Fig. 4A). The difference between results presented here and those observed in mouse PMNs after exposure *in vivo* may be due to the difference in exposure regimens (i.e., *in vivo* versus *in vitro*) or the choice of species. In addition, although [<sup>3</sup>H]TCDD bound specifically to cytosol from rat PMNs, indicating the presence of Ah receptors that bind ligand, it is not known whether these receptors are functional.

It is unlikely that 2,2',4,4'-tetrachlorobiphenyl in the Aroclor mixture accounted entirely for the effects observed with Aroclor 1242 because 2,2',4,4'-tetrachlorobiphenyl was no more potent than the mixture. It is probable that alterations in PMN function after exposure to Aroclor 1242 are at least partly due to non-coplanar PCB congeners present in the mixture and also to additive, cooperative, or synergistic effects of congeners.

Although exposure to PCBs enhanced the respiratory burst of PMNs *in vitro*, the effects on degranulation were more complex. In unstimulated PMNs and in PMNs treated with a subthreshold concentration of fMLP, exposure to Aroclor 1242 caused release of  $\beta$ -glucuronidase from the cells. However, Aroclor 1242 attenuated fMLP-induced enzyme release. fMLP binds to a specific receptor to stimulate degranula-

tion, and one possible explanation for the effects seen with Aroclor 1242 is that it acts as a weak or partial agonist at the fMLP receptor. Alternatively, the Aroclor 1242-induced stimulation of degranulation in quiescent PMNs and the inhibition of fMLP-induced activation may occur by two different mechanisms. These effects occurred only at a concentration of Aroclor 1242 at which cytotoxicity was observed, and it cannot be ruled out that cytotoxicity to the PMNs contributed to inhibition of degranulation.

As with effects on generation of O<sub>2</sub><sup>-</sup>, effects of PCBs on degranulation do not appear to be mediated through the Ah receptor, because 3,3',4,4'-tetrachlorobiphenyl was ineffective but 2,2',4,4'-tetrachlorobiphenyl produced effects similar to Aroclor 1242. 2,2',4,4'-Tetrachlorobiphenyl may contribute to the effects observed with Aroclor 1242, as 2,2',4,4'-tetrachlorobiphenyl was more potent than Aroclor 1242 in inhibiting degranulation. The possibility that other congeners in the mixture or additive or synergistic effects among congeners contribute to the responses in PMNs cannot be ruled out.

In summary, exposure to PCBs *in vitro* alters PMN function in a complex manner. Aroclor 1242 enhances generation of O<sub>2</sub><sup>-</sup> in quiescent and activated PMNs and stimulates degranulation in quiescent cells, but it attenuates fMLP-induced degranulation. 2,2',4,4'-Tetrachlorobiphenyl produces the same pattern of effects as Aroclor 1242, but the coplanar congener 3,3',4,4'-tetrachlorobiphenyl does not affect PMN function *in vitro*. These results suggest that the observed effects of PCBs on rat PMN function *in vitro* are not Ah receptor dependent.

Similar changes in PMN function after exposure to PCBs *in vivo* could contribute to altered response to infection. Recent findings with bacterial endotoxin-induced toxicity support this hypothesis. PMNs play a central role in tissue injury due to endotoxin in liver (25) and lung (26), and treatment with PCBs (2,27) or TCDD (28) increases sensitivity to endotoxin. It is not known whether the increased sensitivity to endotoxin is mediated through PMNs, but the possibility remains that alterations in PMN function caused by PCBs *in vivo* could affect the response to subsequent exposure to pathogens.

## REFERENCES

1. Silkworth JB, Grabstein EM. Polychlorinated biphenyl immunotoxicity: dependence on isomer planarity and the Ah gene complex. *Toxicol Appl Pharmacol* 65:109-115(1982).
2. Thomas PT, Hinsdill RD. Effect of polychlorinated biphenyls on the immune responses of rhesus monkeys and mice. *Toxicol Appl Pharmacol* 44:41-51(1978).

3. Davis D, Safe S. Dose-response immunotoxicities of commercial polychlorinated biphenyls (PCBs) and their interaction with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Toxicol Lett* 48:35–43 (1989).
4. Elo O, Vuojolahti P, Janhunen H, Rantanen J. Recent PCB accidents in Finland. *Environ Health Perspect* 60:315–319 (1985).
5. Chang K-J, Hsieh K-H, Lee T-P, Tang S-Y, Tung T-C. Immunologic evaluation of patients with polychlorinated biphenyl poisoning: determination of lymphocyte subpopulations. *Toxicol Appl Pharmacol* 61:58–63 (1981).
6. Raulf M, Konig W. Modulation of leukotriene generation from human polymorphonuclear granulocytes by polychlorinated biphenyls (PCB). *Immunology* 73:485–490 (1991).
7. Safe S, Bandiera S, Sawyer T, Robertson L, Safe L, Parkinson A, Thomas PE, Ryan DE, Reik LM, Levin W, Denomme MA, Fujita T. PCBs: structure-function relationships and mechanism of action. *Environ Health Perspect* 60:47–56 (1985).
8. Safe S, Bandiera S, Sawyer T, Zmudzka B, Mason G, Romkes M, Fujita T. Effects of structure on binding to the 2,3,7,8-TCDD receptor protein and AHH induction—halogenated biphenyls. *Environ Health Perspect* 61:21–33 (1985).
9. Hewett JA, Roth RA. Dieldrin activates rat neutrophils *in vitro*. *Toxicol Appl Pharmacol* 96:269–278 (1991).
10. Roth RA, Ball TM. Technical grade but not recrystallized alpha-naphthylthiourea potentiates superoxide release by rat neutrophils stimulated *in vitro* by phorbol myristate acetate. *Fundam Appl Toxicol* 7:324–328 (1986).
11. Babior BM, Kipnes RS, Curnutte JT. Biological defense mechanisms. The production by leukocytes of superoxide, a potential bactericidal agent. *J Clin Invest* 52:741–744 (1973).
12. Fishman WH, Springer B, Brunetti R. Application of an improved glucuronidase assay method to the study of human blood  $\beta$ -glucuronidase. *J Biol Chem* 173:449–456 (1948).
13. Bergmeyer HV, Bernt E. Lactate dehydrogenase UV assay with pyruvate and NADH. In: *Methods of enzymatic analysis* (Bergmeyer HV, ed). New York:Academic Press, 1974; 524–579.
14. Helfferich WG, Denison MS. Ultraviolet photo-products of tryptophan can act as dioxin agonists. *Mol Pharmacol* 40:674–678 (1991).
15. Ackerman MF, Gasiewicz TA, Lamm KR, Germolec DR, Luster MI. Selective inhibition of polymorphonuclear neutrophil activity by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Toxicol Appl Pharmacol* 101:470–480 (1989).
16. Heyworth PG, Badwey JA. Protein phosphorylation associated with the stimulation of neutrophils. Modulation of superoxide production by protein kinase C and calcium. *J Bioenerg Biomembr* 22(1):1–26 (1990).
17. Bombick DW, Jankun J, Tullis K, Matsumura F. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin causes increases in expression of *c-erb-A* and levels of protein-tyrosine kinases in selected tissues of responsive mouse strains. *Proc Natl Acad Sci USA* 85:4128–4132 (1988).
18. Bombick DW, Madhukar BV, Brewster DW, Matsumura F. TCDD (2,3,7,8-tetrachlorodibenzo-*p*-dioxin) causes increases in protein kinases particularly protein kinase C in the hepatic plasma membrane of the rat and the guinea pig. *Biochem Biophys Res Commun* 127(1):296–302 (1985).
19. Carrier F, Owens RA, Nebert DW, Puga A. Dioxin-dependent activation of murine *Cyp1a-1* gene transcription requires protein kinase C-dependent phosphorylation. *Mol Cell Biol* 12(4):1856–1863 (1992).
20. Tryphonas H, Luster MI, Schiffman G, Dawson LL, Hodgen M, Germolec D, Hayward S, Bryce F, Loo JCK, Mandy F, Arnold DL. Effect of chronic exposure of PCB (Aroclor 1254) on specific and nonspecific immune parameters in the Rhesus (*Macaca mulatta*) monkey. *Fundam Appl Toxicol* 16:773–786 (1991).
21. Vos JG, Van Loveren H, Schuurman H-J. Immunotoxicity of dioxin: immune function and host resistance in laboratory animals and humans. In: *Biological basis for risk assessment of dioxins and related compounds* (Gallo MA, Scheuplein RJ, Van Der Heijden KA, eds), Banbury Report 35. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, 1991; 79–93.
22. Clark DA, Gaudie J, Szewczuk MR, Sweeney G. Enhanced suppressor cell activity as a mechanism of immunosuppression by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (41275). *Proc Soc Exp Biol Med* 168:290–299 (1981).
23. Tucker AN, Vore SJ, Luster MI. Suppression of B cell differentiation by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Mol Pharmacol* 29:372–377 (1986).
24. Thigpen JE, Faith RE, McConnell EE, Moore JA. Increased susceptibility to bacterial infection as a sequela of exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Infect Immun* 12:1319–1324 (1975).
25. Hewett JA, Schultze AE, VanCise S, Roth RA. Neutrophil depletion protects against liver injury from bacterial endotoxin. *Lab Invest* 66(3):347–361 (1992).
26. Brigham KL, Meyrick B. Interactions of granulocytes with the lungs. *Circ Res* 54(6):623–635 (1984).
27. Shedlofsky SI, Hoglen NC, Rodman LE, Honchel R, Robinson FR, Swim AT, McClain CJ, Robertson LW. 3,3',4,4'-Tetrabromobiphenyl sensitizes rats to the hepatotoxic effects of endotoxin by a mechanism that involves more than tumor necrosis factor. *Hepatology* 14:1201–1208 (1991).
28. Rosenthal GJ, Lebetkin E, Thigpen JE, Wilson R, Tucker AN, Luster MI. Characteristics of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin induced endotoxin hypersensitivity: association with hepatotoxicity. *Toxicology* 56:239–251 (1989).

Volume 101, Supplement 4, September 1993

## Health Effects Institute Environmental Epidemiology Planning Project

This issue of *Environmental Health Perspectives Supplements* "Health Effects Institute Environmental Epidemiology Planning Project," September 1990 – September 1992, presents papers from four selected areas of epidemiologic research: electric and magnetic fields, indoor air pollution and other complex mixtures, tropospheric ozone, and methodologic issues. The Health Effects Institute dedicates the Epidemiology Planning Project documents to the memory of Dr. Richard Remington, former member of the Project Steering Committee and Chairman of the HEI Research Committee from 1989 until June 1992.

To order your copy, contact:  
Government Printing Office, Washington, DC 20402  
or call 202-512-2406.

Environmental Health  
**perspectives**  
Supplements